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(71) Applicant: PROTEIN TECHNOLOGIES  
INTERNATIONAL, INC.  
Saint Louis, Missouri 63164 (US)

(72) Inventors:  
• Waggle, Doyle H.  
Kirkwood, Missouri 63122 (US)  
• Bryan, Barbara A.  
University City, Missouri 63130 (US)

(74) Representative: Tubby, David George  
MARKS & CLERK,  
57-60 Lincoln's Inn Fields  
London WC2A 3LS (GB)

### (54) Recovery of isoflavones from soy molasses

(57) Methods for recovering isoflavones and derivatives thereof from soy molasses are disclosed. In a first embodiment, a method is disclosed in which isoflavones are recovered without any significant conversion of isoflavone conjugates to other forms. In a second embodiment, a method is disclosed whereby isoflavone conju-

gates are converted to glucosides while in the soy material prior to their recovery. In a third embodiment, a method is disclosed in which isoflavones are converted to their aglucone form while in the soy material and prior to their recovery. Also disclosed are various isoflavone enriched products obtained from soy molasses.

**Description**

The present invention relates to processes for recovering isoflavones from soy molasses. In addition, the invention relates to the resulting products containing isoflavones.

5 Isoflavones occur in a variety of leguminous plants, including vegetable protein materials such as soybeans. These compounds include daidzin, 6"-OAc daidzin, 6"-OMal daidzin, daidzein, genistin, 6"-OAc genistin, 6"-OMal genistin, genistein, glycitin, 6"-OAc-glycitin, 6"-OMal-glycitin, glycitein, biochanin A, formononetin, and coumestrol. Typically these compounds are associated with the inherent, bitter flavor of soybeans.

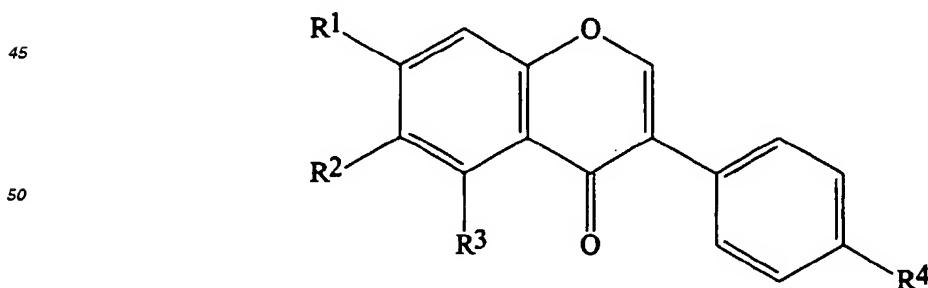
10 The isoflavones in soybean materials include isoflavone glucosides (glucones), isoflavone conjugates and aglucone isoflavones. Isoflavone glucosides have a glucose molecule attached to an isoflavone moiety. Isoflavone conjugates have additional moieties attached to the glucose molecule of an isoflavone glucoside, for example, 6"-OAc genistin contains an acetate group attached to the six position of the glucose molecule of genistin. Aglucone isoflavones consist solely of an isoflavone moiety.

15 Soy contains three "families" of isoflavone compounds having corresponding glucoside, conjugate, and aglucone members: the genistein family, the daidzein family, and the glycitein family. The genistein family includes the glucoside genistin; the conjugates 6"-OMal genistin (6"-malonate ester of genistin) and 6"-OAc genistin (6"-acetate ester of genistin); and the aglucone genistein. The daidzein family includes the glucoside daidzin; the conjugates 6"-OMal daidzin and 6"-OAc daidzin; and the aglucone daidzein. The glycitein family includes the glucoside glycitin; the conjugate 6"-OMal glycitin; and the aglucone glycitein.

20 In the production of commercial products, such as vegetable protein concentrates, the focus has been to remove these materials. For example, in a conventional process for the production of a soy protein concentrate in which soy flakes are extracted with an aqueous acid or an aqueous alcohol to remove water soluble materials from the soy flakes, much of the isoflavones are solubilized in the extract. The extract of water soluble materials, including the isoflavones, is soy molasses. The soy molasses is a byproduct material in the production of soy protein concentrate which is typically 25 discarded. Soy molasses, therefore, is an inexpensive and desirable source of isoflavones, provided that the isoflavones can be separated from the soy molasses.

It has recently been recognized that the isoflavones contained in vegetable protein materials such as soybeans have medicinal value. The aglucone isoflavones are of particular interest. Genistein and daidzein may significantly 30 reduce cardiovascular risk factors. "Plant and Mammalian Estrogen Effects on Plasma Lipids of Female Monkeys", Circulation, vol. 90, p. 1259 (Oct 1994). Genistein and daidzein are also thought to reduce the symptoms of conditions caused by reduced or altered levels of endogenous estrogen in women, such as menopause or premenstrual syndrome. Further, it has recently been recognized that aglucone isoflavones may inhibit the growth of human cancer cells, such 35 as breast cancer cells and prostate cancer cells, as described in the following articles: "Genistein Inhibition of the Growth of Human Breast Cancer Cells, Independence from Estrogen Receptors and the Multi-Drug Resistance Gene" by Peterson and Barnes, Biochemical and Biophysical Research Communications, Vol. 179, No. 1, pp. 661-667, Aug. 30, 1991; "Genistein and Biochanin A Inhibit the Growth of Human Prostate Cancer Cells but not Epidermal Growth Factor Receptor Tyrosine Autophosphorylation" by Peterson and Barnes, The Prostate, Vol. 22, pp. 335-345 (1993); and "Soybeans Inhibit Mammary Tumors in Models of Breast Cancer" by Barnes, et al., Mutagens and Carcinogens in the Diet, pp. 239-253 (1990).

40 The aglucone isoflavones have the following general formula:



wherein, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> may be selected from the group consisting of H, OH and OCH<sub>3</sub>.

Genistein has the formula above where R<sup>1</sup>=OH, R<sup>2</sup>=H, R<sup>3</sup>=OH, and R<sup>4</sup>=OH, daidzein has the formula above where R<sup>1</sup>=OH, R<sup>2</sup>=H, R<sup>3</sup>=H, and R<sup>4</sup>=OH, and glycitein has the formula above where R<sup>1</sup>=OH, R<sup>2</sup>=OCH<sub>3</sub>, R<sup>3</sup>=H, and R<sup>4</sup>=OH.

It is therefore to the isoflavones and to the recovery of an isoflavone enriched material from soy molasses to which the present invention is directed. The present invention is further directed to isoflavone glucosides and aglucone isoflavones - to the conversion of isoflavones of soy molasses to isoflavone glucosides and aglucone isoflavones, and to the recovery of an isoflavone glucoside enriched material and an aglucone isoflavone enriched material from soy molasses.

A general process for converting vegetable protein isoflavone conjugates to aglucone isoflavones is known, and is provided in the currently pending application U.S. Ser. No. 08/477,102 filed June 7, 1995 owned by the assignee of the present application.

Other processes are known in the art for converting isoflavone glucosides to aglucone isoflavones, such as described in Japanese Patent Application 258,669 to Obata, et al. Such processes do not provide for the recovery of an isoflavone enriched material from soy molasses. Such processes also do not provide for the conversion of isoflavone conjugates to isoflavone glucosides or to aglucone isoflavones. Furthermore, these processes achieve only a moderate extent of conversion of isoflavone glucosides to aglucone isoflavones, and require a substantial period of time to effect this moderate extent conversion.

It is therefore an object of the present invention to provide an isoflavone enriched material and a process for producing the same from soy molasses.

It is a further object of the present invention to provide an isoflavone glucoside enriched material and a process for producing the same from soy molasses.

It is still a further object of the present invention to provide an aglucone isoflavone enriched material and a process for producing the same from soy molasses.

The present invention is an isoflavone enriched material and a process for recovering the same from a soy molasses material containing isoflavones. The method comprises providing a soy molasses material containing isoflavones, and separating a cake from the soy molasses material at a pH and a temperature sufficient to cause a majority of the isoflavones to be contained in the cake. Preferably the pH is about 3.0 to about 6.5 and the temperature is about 0°C to about 35°C during the separation. The cake is an isoflavone enriched material.

In one embodiment, a glucoside enriched isoflavone material is formed from the cake of isoflavone enriched material. An aqueous slurry is formed of the isoflavone enriched material. The slurry is treated at a temperature of about 2°C to about 120°C and a pH of about 6 to about 13.5 for a time sufficient to convert isoflavone conjugates in the isoflavone enriched material to isoflavone glucosides. A cake of isoflavone glucoside enriched material may then be separated from the slurry.

In another embodiment, an aglucone isoflavone enriched material is formed from the cake of isoflavone enriched material. An aqueous slurry is formed of the isoflavone enriched material. The slurry is treated at a temperature of about 2°C to about 120°C and a pH of about 6 to about 13.5 for a time sufficient to convert isoflavone conjugates in the isoflavone enriched material to isoflavone glucosides. An enzyme capable of cleaving 1,4-glucoside bonds is contacted with the isoflavone glucosides in the slurry at a temperature of about 5°C to about 75°C and a pH of about 3 to about 9 for a time sufficient to convert the isoflavone glucosides to aglucone isoflavones. A cake of aglucone isoflavone enriched material may be separated from the slurry.

In another aspect, the present invention is an isoflavone glucoside enriched material and a process for recovering the same from soy molasses. The soy molasses is treated at a temperature of from about 2°C to about 120°C and at a pH value of between about 6 to about 13.5 for a time period sufficient to convert isoflavone conjugates contained in the soy molasses to isoflavone glucosides. A cake of isoflavone glucoside enriched material is separated from the soy molasses material at a pH and a temperature sufficient to cause a majority of the isoflavone glucosides to be contained in the cake.

In another aspect, the present invention is an aglucone isoflavone enriched material, and a process for recovering the same from soy molasses. The soy molasses is treated at a temperature of from about 2°C to about 120°C and at a pH value of between about 6 to about 13.5 for a time period sufficient to convert isoflavone conjugates contained in the soy molasses to isoflavone glucosides. An enzyme capable of cleaving 1,4-glucoside bonds is contacted with the isoflavone glucosides in the soy molasses material at a temperature of about 5°C to about 75°C and a pH of about 3 to about 9 for a time period sufficient to convert the isoflavone glucosides to aglucone isoflavones. A cake of aglucone isoflavone enriched material is separated from the soy molasses material at a pH and a temperature sufficient to cause a majority of the aglucone isoflavones to be contained in the cake.

The starting material for the processes described herein is soy molasses. Soy molasses is a by-product of many commercial processes involving soybeans or soybean derivatives, such as processes for producing protein extracts, protein whey, and protein concentrate products. Accordingly, soy molasses is produced in large quantities to such an extent that soy molasses is a relatively inexpensive commodity.

Soy molasses is generally considered to be the soy solubles removed from soy insolubles by washing with alcohol or acid. Soy molasses is typically an aqueous mixture containing about 50% or more solids that comprise about 6% (based on the total weight of the soy molasses) protein, about 3% ash, about 5% fat, and about 36% carbohydrates.

Soy molasses is also known in the art as soya bean solubles. "Soy molasses material" as that term is used herein refers to a composition containing soy molasses, and/or derivatives of soy molasses such as an isoflavone glucoside enriched soy molasses material and an aglucone isoflavone enriched soy molasses material. Accordingly, these terms are used interchangeably herein.

5 In a preferred embodiment, the soy molasses starting material is produced from defatted soy flakes from which oil has been removed by solvent extraction in a conventional manner. The defatted soy flakes are extracted with water which has been adjusted to an acidic pH, preferably about pH 4 to about pH 5, by the addition of one or more suitable acids such as acetic acid, sulfuric acid, phosphoric acid, hydrochloric acid or any other suitable reagent. Preferably the ratio of the acidic extractant to soy flakes is about 16:1 to about 20:1 by weight. To improve the efficacy of the  
10 extraction, the temperature of the extractant may be elevated above room temperature, preferably between about 32°C and about 55°C. After the extraction, the soy molasses extract is removed from the soy insolubles.

In another embodiment, defatted soy flakes are extracted with aqueous alcohol to produce the soy molasses starting material. Preferably, the flakes are extracted with about 80% aqueous ethanol at a ratio of about 16:1 to about 20:1 by weight of extractant to soy flakes. The temperature of the alcohol extractant may be elevated above room  
15 temperature, preferably from about 32°C to about 55°C, to improve the efficacy of the extraction. The soy molasses extract is then removed from the soy insolubles to provide the soy molasses starting material.

An isoflavone enriched material may be recovered from the soy molasses starting material. The soy molasses material may be diluted with water to a solids content of from about 6% to about 13%, with 13% being most preferred. Dilution of the soy molasses material is not a requirement for the process, however, diluting the relatively thick soy  
20 molasses material facilitates processing the material.

The soy molasses material, preferably diluted, is treated at a pH and a temperature at which a majority of the isoflavones will separate from the soy molasses upon performing a separation procedure. In a preferred embodiment, the soy molasses material is treated at a pH of about 3.0 to about 6.5 and a temperature of about 0°C to about 35°C to maximize the insolubility of the isoflavones in the soy molasses. Isoflavones may be separated from the soy molasses  
25 at pH values outside the preferred range and at temperatures above 35°C, however, these conditions are less preferred since less isoflavones are separated from the soy molasses in the separation procedure. The pH of the soy molasses may be adjusted with a suitable conventional acidic or basic reagent, if necessary. It is most preferred that the pH of the soy molasses be adjusted to about 4.5. It is also preferred to chill or cool the soy molasses to a temperature of about 0°C to about 10°C, and most preferably to a temperature of about 4°C to about 7°C.

30 The soy molasses material is then subjected to a separation procedure to separate a cake of isoflavone enriched material from the soy molasses material. The separation is performed while the soy molasses material is maintained under the previously described pH and temperature conditions.

In one embodiment, the cake of isoflavone enriched material is separated by centrifuging the soy molasses and decanting the supernatant from the cake. Centrifugation is preferably performed at about 3,000 to about 10,000 rpm for approximately 30 minutes at about 0°C to about 10°C.  
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In another embodiment, the isoflavone enriched cake may be separated from the soy molasses material by filtration. Preferably the filtration is done at the previously described pH and temperature conditions, most preferably at a pH of about 4.5 and a temperature of about 0°C to about 10°C.

40 In another aspect of the present invention, a cake of isoflavone glucoside enriched material may be recovered from soy molasses. A soy molasses starting material is obtained as described above. Although not a requirement, it is preferred that the soy molasses material be diluted to a solids content of from about 6% to about 13%, and most preferably about 13%, to facilitate processing the material.

A conversion operation is then performed on the soy molasses to convert isoflavone conjugates in the soy molasses to isoflavone glucosides. A substantial portion of the isoflavones in the soy molasses are isoflavone conjugates, therefore the conversion substantially increases the amount of isoflavone glucosides in the soy molasses material. The conversion has been found to be dependent on the pH and the temperature of the soy molasses.  
45

The pH range for conversion of the isoflavone conjugates to isoflavone glucosides in soy molasses is from about 6 to about 13.5. The pH of the soy molasses should be adjusted to the desired pH, if necessary, with a suitable base, caustic agent, or basic reagent if the pH is to be raised, or, if the pH is to be lowered, with a suitable acid or acid reagent.  
50 The conversion of isoflavone conjugates to isoflavone glucosides has been found to be base catalyzed, and so it is most preferred to utilize a high pH to achieve rapid conversion. The most preferred pH for conversion of the isoflavone conjugates to isoflavone glucosides is a pH of about 9 to about 11.

The temperature range for conversion of the isoflavone conjugates to isoflavone glucosides in soy molasses is from about 2°C to about 120°C. The temperature range at which the conversion readily occurs depends on the pH of the soy molasses material. The inventors have found that the conversion occurs easily at lower temperatures when the pH is relatively high. For example, at a pH of about 11 the conversion occurs rapidly and efficiently at a temperature range of about 5°C to about 50°C. At a pH of about 9 conversion occurs efficiently within a temperature range of about 45°C to about 75°C. When the pH of the soy molasses is relatively low, the conversion occurs at higher temperatures.  
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For example, at a pH of about 6, the conversion occurs within a temperature range of about 80°C to about 120°C. In a preferred embodiment, the conversion is effected at about 35°C and a pH of about 11. In another preferred embodiment, the conversion is effected at a temperature of about 73°C and a pH of about 9.

The time period required for conversion of isoflavone conjugates to isoflavone glucosides depends primarily upon the pH and temperature range utilized in the soy molasses. Such conversion times typically range from about 15 minutes up to several hours or longer. Conversion occurs more rapidly at a higher pH and at a higher temperature. At a pH of about 9, conversion is substantially complete in about 4 hours to about 6 hours at 73°C. In a most preferred embodiment, the isoflavone conjugates are converted to isoflavone glucosides in about 30 minutes to about 1 hour, preferably about 45 minutes, at a pH of about 11 and at a temperature of about 35°C.

The conversion of the isoflavone conjugates to isoflavone glucosides is remarkably efficient, converting at least a majority, and preferably substantially all of the isoflavone conjugates present to isoflavone glucosides. The term, a "majority" refers to an extent of conversion of at least about 50%. The term "substantially, all" refers to an extent of conversion of at least about 80%, and most preferably at least about 90%.

Following the conversion of the isoflavone conjugates to isoflavone glucosides, a cake of isoflavone glucoside enriched material may be separated from the soy molasses material. The soy molasses material is treated at a pH and a temperature at which a majority of the isoflavone glucosides will separate from the soy molasses material in a separation procedure. In a preferred embodiment the soy molasses material is maintained at a pH of about 3 to about 6.5, most preferably about 4.5, and at a temperature of about 0°C to about 35°C, preferably about 0°C to about 10°C, and most preferably about 4°C to about 7°C, during the separation process. The pH of the soy molasses material may be adjusted with a suitable conventional acidic or basic reagent, if necessary.

The separation may be effected by conventional means for separating solids from a liquid. The isoflavone glucoside enriched cake is preferably separated by centrifugation or filtration as described above with respect to separating an isoflavone enriched cake from a soy molasses material.

In yet another aspect of the invention, a cake of aglucone isoflavone enriched material may be recovered from soy molasses. A soy molasses starting material is obtained as described above, preferably diluted with water to a solids content of about 6% to about 13%. The soy molasses starting material is treated to convert the isoflavone conjugates to isoflavone glucosides as described above.

An enzymatic conversion operation is then performed on the isoflavone glucoside enriched soy molasses material by contacting a suitable enzyme with isoflavone glucosides in the soy molasses material at a suitable pH and temperature to convert the isoflavone glucosides to aglucone isoflavones. The two-step conversion process effectively converts substantially all of the isoflavone conjugates and isoflavone glucosides in the soy molasses material to aglucone isoflavones, substantially increasing the amount of aglucone isoflavones in the soy molasses material.

The conversion of isoflavone glucosides to aglucone isoflavones has been found to be dependent on a variety of factors including the type of enzymes present in the soy molasses material, distribution of enzyme concentrations, activities of the enzymes, and the pH and temperature of the soy molasses material during the conversion. The enzymes required to effect the conversion are enzymes capable of cleaving the glucosidic linkage between the isoflavone moiety and the glucose molecule of the isoflavone glucosides. In a preferred embodiment, the enzymes are saccharidase, esterase, or gluco-amylase enzymes capable of cleaving 1,4-glucoside bonds. The enzymes may be inherently present in the soy molasses material, or may be commercially available enzymes which are added to the soy molasses material. Inherently present enzymes are referred to herein as "residual" enzymes, and enzymes that are added to the soy molasses are referred to herein as "supplemental" enzymes.

Sufficient enzyme should be present in the soy molasses material to convert at least a majority, and preferably substantially all, of the isoflavone glucosides to aglucone isoflavones. Generally, if the residual enzymes in the soy molasses material are insufficient to effect the conversion, supplemental enzymes should be added to the soy molasses material. In a preferred embodiment, supplemental enzymes are added to the soy molasses material regardless of whether sufficient residual enzymes are present in the soy molasses material since addition of supplemental enzymes dramatically decreases the time necessary to effect substantially complete conversion of the glucosides to aglucones. If supplemental enzymes are added, the supplemental enzymes should be added so that the total concentration of enzyme present is about 0.1% to about 10% by weight of the solids in the soy molasses material on a dry basis.

Supplemental enzymes are selected based on optimum activity at selected pH and temperature conditions, and cost effectiveness. The supplemental enzymes are enzymes capable of cleaving the bond between the isoflavone moiety and the glucose molecule of the isoflavone glucosides, such as saccharidase, esterase, and gluco-amylase enzymes capable of cleaving 1,4-glucoside bonds. Preferred supplemental enzymes are commercially available alpha- and beta-glucosidase enzymes, beta-galactosidase enzymes, gluco-amylase enzymes, and pectinase enzymes. Particularly preferred are enzymes such as Biopectinase 100L (which is preferably utilized at a pH range of from about 3 to about 6), Biopectinase 300L (optimum pH range from about 3 to about 6), Biopectinase OK 70L (optimum pH range from about 3 to about 6), Biolactase 30,000 (optimum pH range from about 3 to about 6) Neutral Lactase (optimum pH range from about 6 to about 8), all of which are available from Quest International, 1833 57th Street, Post Office

Box 3917, Sarasota, Florida 34243. Also especially preferred are Lactase F (which is preferably utilized at a pH range of from about 4 to about 6), and Lactase 50,000 (optimum pH range from about 4 to about 6), both available from Amano International Enzyme Co., Inc., Post Office Box 1000, Troy, Virginia 22974. Other particularly preferred supplemental enzymes include G-Zyme G990 (optimum pH from about 4 to about 6) and Enzeco Fungal Lactase Concentrate (optimum pH from about 4 to about 6) available from Enzyme Development Corporation, 2 Penn Plaza, Suite 2439, New York, New York 10121; Lactozyme 3000L (which preferably is utilized at a pH range from about 6 to about 8), and Alpha-Gal 600L (which preferably is utilized at a pH range of from about 4 to about 6.5), available from Novo Nordisk Bioindustrials, Inc., 33 Turner Road, Danbury, Connecticut 06813; Maxilact L2000 (which is preferably utilized at a pH range of from about 4 to about 6), available from Gist Brocades Food Ingredients, Inc., King of Prussia, Pennsylvania, 19406; and Neutral Lactase (which is preferably utilized at a pH range of from about 6 to about 8), available from Pfizer Food Science Group, 205 East 42nd Street, New York, New York 10017.

The pH range for conversion of the isoflavone glucosides to aglucone isoflavones is from about 3 to about 9. The pH that is utilized depends primarily upon the type of enzyme used, and should be selected accordingly. The residual enzyme is active within a pH range of about 7 to about 9, although it is believed that the pH of the soy molasses material is lowered during the course of the conversion. The supplemental enzymes are active within an optimum pH range specified by the manufacturer of the enzyme, as shown above for several specific enzymes. Typically the supplemental enzymes are active either in a neutral pH range from about 6 to about 8, or in an acidic pH range from about 3 to about 6.

The pH of the soy molasses material may be adjusted to a desired value for conducting the conversion of isoflavone glucosides to aglucone isoflavones. In most instances the pH is reduced from the relatively high or basic pH required to convert the isoflavone conjugates to isoflavone glucosides by the addition of one or more suitable acids such as acetic acid, sulfuric acid, phosphoric acid, hydrochloric acid, or any other suitable reagent.

The temperature range of the soy molasses material for the conversion of glucosides to aglucones is from about 5°C to about 75°C. The temperature significantly affects the activity of the enzymes, and therefore, the rate of conversion. The supplemental enzymes may be active above 70°C, for example Alpha-Gal 600L is active at 75°C, however, it is preferred to conduct the conversion at lower temperatures to avoid enzyme deactivation. In a preferred embodiment, the conversion is effected between about 35°C and about 45°C.

The time required for conversion of the glucosides to aglucones depends upon enzyme-related factors, particularly concentration, and the temperature and pH of the system. In most instances it is possible to achieve substantially complete conversion within 24 hours, however, it is preferred that supplemental enzyme be added to dramatically increase the rate of the reaction. The selected supplemental enzyme, enzyme concentration, pH and temperature preferably cause substantially complete conversion within about 2 hours, and most preferably within about 1 hour.

The conversion of the isoflavone glucosides to aglucone isoflavones is remarkably efficient, converting at least a majority, and preferably substantially all of the glucosides present to aglucones. The term "a majority" refers to an extent of conversion of at least about 50%. The term "substantially all" refers to an extent of conversion of at least about 80%, and most preferably at least about 90%.

Following the conversion of the isoflavone glucosides to aglucone isoflavones, a cake of aglucone isoflavone enriched material may be separated from the soy molasses material. The soy molasses material is treated at a pH and a temperature at which a majority of the aglucone isoflavones will separate from the soy molasses material in a separation procedure. Preferably, the soy molasses material is maintained at a pH of about 3 to about 6.5, most preferably about 4.5, and at a temperature of about 0°C to about 35°C, preferably about 0°C to about 10°C, and most preferably about 4°C to about 7°C, during the separation process. The pH of the soy molasses material may be adjusted with a suitable conventional acidic or basic reagent, if necessary.

The separation may be effected by conventional means for separating solids from a liquid. The aglucone isoflavone enriched cake is preferably separated by centrifugation or filtration as described above with respect to separating an isoflavone enriched cake from a soy molasses material.

An aglucone isoflavone enriched material may also be produced from an isoflavone enriched material recovered from soy molasses, where the process for recovering an isoflavone enriched material from soy molasses is described above. Water is added to the recovered cake of isoflavone enriched material to form a slurry of the isoflavone enriched material. Preferably the slurry is diluted to about 6% to about 13% solids, although a higher solids content may be used. Isoflavone conjugates in the slurry are then converted to isoflavone glucosides by treating the slurry under the same conditions described above with respect to converting isoflavone conjugates to isoflavone glucosides in soy molasses. In particular, the slurry is treated at a pH of about 6 to about 13.5, preferably about pH 9 to about pH 11, and a temperature of about 2°C to about 120°C for a period of about 15 minutes to several hours. Most preferably the slurry is treated at a pH of about 11 and a temperature of about 5°C to about 50°C, preferably about 35°C, for a period of about 30 minutes to about 1 hour, or at a pH of about 9 and a temperature of about 45°C to about 75°C, preferably about 73°C, for a period of about 4 hours to about 6 hours. If desired, an isoflavone glucoside enriched material may be separated from the slurry in a manner similar to the separation of an isoflavone enriched material from soy molasses described above.

Isoflavone glucosides in the slurry are then converted to aglucone isoflavones under the same conditions described above with respect to converting isoflavone glucosides to aglucone isoflavones in a soy molasses material. In particular, the isoflavone glucosides in the slurry are contacted with an enzyme capable of cleaving the glucosidic linkage between the isoflavone moiety and the glucose molecule of the isoflavone glucosides under suitable pH and temperature conditions for a period of time sufficient to convert the isoflavone glucosides to aglucone isoflavones. Preferred enzymes, pH conditions, temperatures, and time periods are described above. An aglucone isoflavone enriched material may be separated from the slurry in a manner similar to the separation of an isoflavone enriched material from soy molasses described above.

An aglucone isoflavone enriched material may also be produced from an isoflavone glucoside enriched material recovered from a soy molasses material, where the process for recovering an isoflavone glucoside enriched material from a soy molasses material is described above. Water is added to the recovered cake of isoflavone glucoside enriched material to form a slurry of the isoflavone glucoside enriched material. Preferably the slurry is diluted to about 6% to about 13% solids, although a higher solids content may be used. The isoflavone glucosides in the slurry are converted to aglucone isoflavones in the same manner described above with respect to the isoflavone glucoside enriched slurry formed from an isoflavone enriched slurry. An aglucone isoflavone enriched material may be separated from the slurry after the conversion in a manner similar to the separation of an isoflavone enriched material from soy molasses described above.

#### Experimental

The present invention is illustrated in more detail by the following examples. The examples are intended to be illustrative, and should not be interpreted as limiting or otherwise restricting the scope of the invention in any way.

As noted above, soy materials, including soy molasses, include the genistein, daidzein, and glycinein "families" of isoflavones having corresponding glucoside, conjugate, and aglucone members, where the genistein family contains the conjugates 6"-OMal genistin, 6"-OAc genistin, the glucoside genistin, and the aglucone genistein; the daidzein family contains the conjugates 6"-OMal daidzin, 6"-OAc daidzin, the glucoside daidzin, and the aglucone daidzein; and the glycinein family includes the conjugate 6"-OMal glycitin, the glucoside glycitin, and the aglucone glycinein. In the following examples the relative concentrations of isoflavones are measured either as a total concentration of an isoflavone family, or as individual percentages of each isoflavone in a family of isoflavones. For example, the total concentration of the genistein family of isoflavones is the sum of the concentrations of 6"-OMal genistin, 6"-OAc genistin, genistin, and genistein, and the percentage of each of the isoflavones in the genistein family is determined relative to the other genistein family isoflavones: % genistin + % 6"OMal genistin + % 6" OAc genistin + % genistein = 100%.

#### EXAMPLE 1

In a first experiment, the recovery of an isoflavone enriched material from soy molasses is examined at various concentrations of soy molasses. The total concentration of each isoflavone family is measured in a soy molasses sample having a selected concentration, in a cake separated from the soy molasses sample according to the method of the invention, and in the liquid whey from which the cake is removed by the separation procedure.

Soy molasses is analyzed for the total concentration of all forms of isoflavones present. Samples of the soy molasses are diluted with water to a solids content of 28% (1:2 dilution), 13.7% (1:4 dilution), and 6.6% (1:8 dilution). All samples are pH adjusted to 4.5. The treated samples are then centrifuged at a rate of between 3000 rpm for 30 minutes to separate and produce liquid whey and cake portions from the samples. One set of samples is centrifuged at a temperature of 0.6°C. Samples having 28% and 13.7% soy molasses solids are centrifuged at a temperature of 60°C for comparison with samples having the same concentration of soy molasses solids that are separated at 0.6°C. The resulting liquid and cake portions of the samples are analyzed for the total concentration of all forms of isoflavones present.

Table 1 sets forth the concentrations of isoflavones in the various cake and liquid fractions obtained from the previously described testing. The indicated totals are the totals of all forms of the particular isoflavone including conjugates, glucoside, and aglucone forms expressed in mg of isoflavone per gram of cake or liquid fraction solids.

TABLE 1

	Sample	Total Genistein (family) mg/g	Total Daidzein (family) mg/ g	Total Glycitein (family) mg/ g
5	Soy Molasses Starting Material Not separated	6.1	4.8	1.0
10	1:2 Dilution (28% solids) Whey separated at 0.6°C	2.8	3.0	0.6
15	1:2 Dilution Cake separated at 0.6°C	16.9	10.9	1.9
20	1:2 Dilution Whey separated at 60°C	3.8	4.0	0.8
25	1:2 Dilution Cake separated at 60°C	14.1	8.2	1.5
30	1:4 Dilution (13.7% solids) Whey separated at 0.6°C	3.0	3.4	0.7
35	1:4 Dilution Cake separated at 0.6°C	18.3	11.0	2.0
40	1:4 Dilution Whey separated at 60°C	4.4	4.3	0.8
45	1:4 Dilution Cake separated at 60°C	13.4	7.2	1.5
50	1:8 Dilution (6.6% solids) Whey separated at 0.6°C	4.3	4.5	0.9
55	1:8 Dilution Cake separated at 0.6°C	20.1	10.2	2.1

In all separated samples, the concentration of isoflavones is significantly higher in the cake than in soy molasses starting material and much higher than the concentration of isoflavones in the liquid whey fraction solids. The samples separated at 0.6°C contained a higher concentration of isoflavones in the cake than corresponding samples separated at 60°C, which had higher concentrations of isoflavones in the whey fraction solids.

#### EXAMPLE 2

In another experiment, the recovery of an isoflavone glucoside enriched material from soy molasses is examined. Isoflavone conjugates in the soy molasses are converted to isoflavone glucosides, and an isoflavone glucoside enriched cake is separated from the soy molasses material. The extent of conversion is determined by the quantitative decrease of the percentage and concentration of malonate and acetate esters of an isoflavone family coupled with a corresponding quantitative increase of the percentage of the glucoside of the same isoflavone family.

Soy molasses starting material is analyzed for concentration of individual isoflavone compounds. Two samples of the soy molasses material are made by diluting the soy molasses with water in the following ratios: 1:4(100g of molasses + 300g water); and 1:8(50g of molasses + 350g water). The pH of the samples is adjusted to 11, and the temperature of the samples is held at 35°C for 30 minutes. The pH of the samples is then adjusted to 4.5 and the temperature is adjusted to 4°C. The samples are centrifuged at 10,000 rpm at 4°C to separate the molasses samples into a cake and a liquid whey. The whey and the cake are analyzed for concentration of individual isoflavone compounds.

Table 2 illustrates the change in the proportions between the various forms of isoflavones resulting from the conversion of isoflavone conjugates to isoflavone glucosides as compared to the soy molasses starting material. Isoflavone concentrations are indicated as parts per million(ppm) within the sample, and as percentages of the total amount of the particular isoflavone (the total of the conjugate, glucoside, and aglucone forms) within the liquid or cake portion.

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TABLE 2a

Sample	Genistin	6"-OMAL-Genistin	6"-OAC-Genistin	Genistein
Soy Molasses ppm % isoflavone	4678 77	1329 22	0 0	88 1
<u>1:4 Dilution Whey</u> ppm % isoflavone	2221 98	17 1	0 0	30 1
<u>1:4 Dilution Cake</u> ppm % isoflavone	28621 99	68 0	0 0	261 1
<u>1:8 Dilution Whey</u> ppm % isoflavone	2852 98	24 1	0 0	36 1
<u>1:8 Dilution Cake</u> ppm % isoflavone	27517 99	101 0	0 0	272 1

TABLE 2b

Sample	Daidzin	6"-OMAL-Daidzin	6"-OAC-Daidzin	Daidzein
Soy Molasses ppm % isoflavone	3533 74	928 20	210 4	84 2
<u>1:4 Dilution Whey</u> ppm % isoflavone	2652 92	179 6	29 1	21 1
<u>1:4 Dilution Cake</u> ppm % isoflavone	16133 97	192 1	0 0	232 1
<u>1:8 Dilution Whey</u> ppm % isoflavone	3356 94	187 5	0 0	27 1

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55

**TABLE 2b (cont.)**

Sample	Daidzin	6"-OMAL-Daidzin	6"-OAC-Daidzin	Daidzein
<u>1:8 Dilution Cake</u> ppm % isoflavone	12617 97	138 1	0 0	245 2

**TABLE 2c**

Sample	Glycitin	6"-OMAL-Glycitin	Glycitein
Soy Molasses ppm % isoflavone	500 52	105 11	360 37
<u>1:4 Dilution Whey</u> ppm % isoflavone	341 92	28 8	0 0
<u>1:4 Dilution Cake</u> ppm % isoflavone	1442 96	0 0	66 4
<u>1:8 Dilution Whey</u> ppm % isoflavone	406 94	28 6	0 0
<u>1:8 Dilution Cake</u> ppm % isoflavone	1146 100	0 0	0 0

In all samples subjected to conditions for conversion of isoflavone conjugates to isoflavone glucosides the percentage of isoflavone glucosides in both cake and liquid portions is significantly higher than in the corresponding unconverted soy molasses sample, and the percentage of corresponding isoflavone conjugates in the samples is significantly lower, demonstrating that a large portion of isoflavone conjugates are converted to their glucoside form. Furthermore, upon separation a large proportion of the glucoside isoflavones are separated in the cake to form an isoflavone glucoside enriched material, as can be seen from the relative concentrations of the soy molasses starting material and the whey and cake portions of each sample.

**EXAMPLE 3**

In another experiment, the conversion of isoflavones to aglucone isoflavones in soy molasses is examined. Isoflavone conjugates in the soy molasses are converted to isoflavone glucosides, and the isoflavone glucosides are then converted to aglucone isoflavones. The extent of conversion of the isoflavone glucosides to aglucone isoflavones is determined by the quantitative decrease of the concentration of the glucoside of an isoflavone family coupled with a corresponding quantitative increase of the percentage of the aglucone of the same isoflavone family.

Soy molasses starting material is diluted 1:4 with water and is analyzed for concentration of individual isoflavone compounds. The pH of the molasses is then adjusted to 11. The soy molasses is held at room temperature for 1 hour to produce a glucoside enriched soy molasses material. The glucoside enriched soy molasses material is analyzed for concentration of individual isoflavone compounds. Four samples are prepared from the glucoside enriched soy molasses material after the pH of the material is adjusted to 4.5. Each sample is inoculated with an enzyme, where the following enzymes are added to the samples, respectively, at 10% by weight of the molasses solids in each sample: G-Zyme 990, Biopectinase 100L, Lactase 50,000, and Alpha-Gal 600L. The samples are then treated at 50°C for 6 hours to form an aglucone isoflavone enriched soy molasses material. The aglucone isoflavone enriched soy molasses is then analyzed for isoflavone content.

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Table 3 illustrates the distribution between the various forms of isoflavones resulting from the previously described testing. Isoflavone concentrations are indicated as parts per million(ppm) within the sample, and as percentages of the total amount of the particular isoflavone (the total of the conjugate, glucoside, and aglucone forms).

TABLE 3a

Sample	Genistin	6"-OMAL-Genistin	6"-OAC-Genistin	Genistein
<u>Soy Molasses</u>				
ppm	4678	1329	0	88
% isoflavone	77	22	0	1
<u>Glucoside rich soy molasses</u>				
ppm	6763	0	0	104
% isoflavone	98	0	0	2
<u>G-Zyme 990, 10%</u>				
ppm	3903	0	0	1993
% isoflavone	66	0	0	44
<u>Biopectinase 100L, 10 %</u>				
ppm	2865	0	0	2919
% isoflavone	50	0	0	50
<u>Lactase 50,000, 10%</u>				
ppm	0	0	0	4601
% isoflavone	0	0	0	100
<u>Alpha-Gal 600L, 10%</u>				
ppm	28	0	0	4566
% isoflavone	1	0	0	99

TABLE 3b

Sample	Daidzin	6"-OMAL-Daidzin	6"-OAC-Daidzin	Daidzein
<u>Soy Molasses</u>				
ppm	3533	928	210	84
% isoflavone	74	20	4	2
<u>Glucoside rich soy molasses</u>				
ppm	4377	0	0	43
% isoflavone	99	0	0	1
<u>G-Zyme 990, 10%</u>				
ppm	840	0	82	2331
% isoflavone	27	0	2	71
<u>Biopectinase 100L, 10%</u>				
ppm	541	0	94	2701
% isoflavone	16		3	81

**TABLE 3b (cont.)**

Sample	Daidzin	6"-OMAL-Daidzin	6"-OAC-Daidzin	Daidzein
<u>Lactase 50,000, 10%</u> ppm % isoflavone	0 0	0 0	92 3	2875 97
<u>Alpha-Gal 600L, 10%</u> ppm % isoflavone	0 0	0 0	89 3	2882 97

**TABLE 3c**

Sample	Glycitin	6"-OMAL-Glycitin	Glycitein
<u>Soy Molasses</u> ppm % isoflavone	500 52	105 11	360 37
<u>Glucoside rich soy molasses</u> ppm % isoflavone	433 100	0 0	0 0
<u>G-Zyme 990, 10%</u> ppm % isoflavone	346 75	0 0	114 25
<u>Biopectinase 100L, 10%</u> ppm % isoflavone	195 45	0 0	237 55
<u>Lactase 50,000, 10%</u> ppm % isoflavone	0 0	0 0	366 100
<u>Alpha-Gal 600L, 10%</u> ppm % isoflavone	0 0	0 0	356 100

The aglucone isoflavone content of the enzymatically treated samples is significantly higher than the soy molasses and the glucoside enriched soy molasses material, indicating that the enzymatic treatment converted substantial amounts of glucoside isoflavones to aglucone isoflavones. Selection of the proper enzyme, enzyme concentration, pH and temperature for the conversion permits conversion of substantially all of the isoflavone glucosides to aglucone isoflavones, as demonstrated by the isoflavone distribution in the Lactase 50,000 and Alpha-Gal 600L samples.

**EXAMPLE 4**

In a final experiment, the isoflavone content of an isoflavone enriched material, an isoflavone glucoside enriched material, and an aglucone isoflavone enriched material is examined and the distribution of the isoflavones in the materials is compared. Soy molasses is diluted to a 1:4 ratio with water. A sample of the diluted soy molasses is adjusted to a pH of 4.5, is chilled to a temperature of 0.6°C in an ice bath for 30 minutes, and is centrifuged at a rate of 3000 rpm for 30 minutes to separate a cake of isoflavone enriched material. The remaining diluted soy molasses is then adjusted to a pH of 11 with sodium hydroxide and is treated at 50°C for 1 hour to convert isoflavone conjugates in the molasses to isoflavone glucosides. A sample of the glucoside enriched molasses is adjusted to a pH of 4.5, is chilled

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to a temperature of 0.6°C in an icebath for 30 minutes, and is centrifuged at a rate of 3000 rpm for 30 minutes to separate a cake of isoflavone glucoside enriched material. The remaining isoflavone glucoside enriched soy molasses material is adjusted to pH 4.5, and the enzyme G-Zyme 990 is added to the material at a concentration of 2.6g enzyme/ 100g of molasses material. The enzyme and the isoflavone glucoside enriched soy molasses material are then treated at 50°C for 18 to 20 hours to convert the isoflavone glucosides to aglucone isoflavones. A sample of the aglucone isoflavone enriched soy molasses material is chilled to a temperature of 0.6°C in an icebath for 30 minutes, and is centrifuged at a rate of 3000 rpm for 30 minutes to separate a cake of aglucone isoflavone enriched material. The recovered cakes of isoflavone enriched material, isoflavone glucoside enriched material, and aglucone isoflavone enriched material are then analyzed for isoflavone content.

Table 4 below shows the distribution of the isoflavones in the cakes of isoflavone enriched material, isoflavone glucoside enriched material, and aglucone isoflavone enriched material. Isoflavone distribution is indicated as percentages of the total amount of the particular isoflavone (the total of the conjugate, glucoside, and aglucone forms).

TABLE 4a

Sample	Genistin	6"-OMAL-Genistin	6"-OAC-Genistin	Genistein
<u>Isoflavone rich material</u>				
% isoflavone	83	16	0	1
<u>Glucoside rich material</u>				
% isoflavone	99	0	0	1
<u>Aglucone rich material</u>				
% isoflavone	3	0	0	97

TABLE 4b

Sample	Daidzin	6"-OMAL-Daidzin	6"-OAC-Daidzin	Daidzein
<u>Isoflavone rich material</u>	81	12	5	1
% isoflavone				
<u>Glucoside rich material</u>	99	0	0	1
% isoflavone				
<u>Aglucone rich material</u>	0	0	0	100
% isoflavone				

TABLE 4c

Sample	Glycitin	6"-OMAL-Glycitin	Glycitein
<u>Isoflavone rich material</u>	40	8	52
% isoflavone			
<u>Glucoside rich material</u>	95	0	5
% isoflavone			
<u>Aglucone rich material</u>	54	18	28
% isoflavone			

The effectiveness of the conversion steps can be seen in the isoflavone distribution of the materials. The isoflavone glucoside enriched material contains significantly higher amounts of isoflavone glucosides than the isoflavone enriched material and the aglucone isoflavone material, having an isoflavone content which is comprised of almost entirely isoflavone glucosides. The aglucone isoflavone enriched material contains significantly higher amounts of aglucone isoflavones than the isoflavone glucoside enriched material and the isoflavone enriched material, having an isoflavone content which is comprised of almost entirely aglucone isoflavones.

In the above examples, all percentages indicated for 6"-OMal-genistin, 6"-OAc-genistin, 6"-OMal-daidzin, 6"-OAc-daidzin, glycitin, 6"-OMal-glycitin, and glycitein are calculated values. The following is a description of a method for quantifying isoflavones in soy products. The isoflavones are extracted from soy products by mixing 0.75 gram of sample (spray dried or finely ground powder) with 50 ml of 80/20 methanol/water solvent. The mixture is shaken for 2 hours

at room temperature with an orbital shaker. After 2 hours, the remaining undissolved materials are removed by filtration through Whatman No. 42 filter paper. Five ml of the filtrate are diluted with 4 ml of water and 1 ml of methanol.

The extracted isoflavones are separated by HPLC (High Performance Liquid Chromatography) using a Hewlett Packard C18 Hypersil reverse phase column. The isoflavones are injected onto the column and eluted with a solvent gradient starting with 88% methanol, 10% water, and 2% glacial acetic acid and ending with 98% methanol and 2% glacial acetic acid. At a flow rate of 0.4 ml/min, all the isoflavones - genistin, 6'-O-acetylgenistin, 6'-O-malonylgenistin, genistein, daidzin, 6'-O-acetyldaidzin, 6'-O-malonyldaidzin, daidzin, glycitin and its derivatives and glycinein - are clearly resolved. Peak detection is by UV absorbance at 260 nm. Identification of the peaks was performed by HPLC-mass spectrometer.

Quantification is achieved by using pure standards (genistin, genistein, daidzin and daidzein) obtained from Indofine Chemical Company, Sommerville, NJ. Response factors (integrated area/concentration) are calculated for each of the above compounds and are used to quantitate unknown samples. For the conjugated forms for which no pure standards are available, response factors are assumed to be that of the parent molecule but corrected for molecular weight difference. The response factor for glycitin is assumed to be that for genistin corrected for molecular weight difference. This method provides the quantities of each individual isoflavone. For convenience, total genistein, total daidzein and total glycinein can be calculated, and represent the aggregate weight of these compounds if all the conjugated forms are converted to their respective unconjugated forms. These totals can also be measured directly by a method using acid hydrolysis to convert the conjugated forms.

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### Claims

1. A process for obtaining an isoflavone enriched material from soy molasses comprising separating said isoflavone enriched material from a soy molasses material containing isoflavones, at a pH and a temperature effective to cause a majority of the isoflavones to separate with the isoflavone enriched material.
2. A process according to claim 1 wherein said isoflavone enriched material is separated from said soy molasses material at a pH of about 3 to about 6.5 and a temperature of about 0°C to about 35°C.
3. A process according to claim 2 wherein said isoflavone enriched material is separated from said soy molasses material at a pH of about 4.5.
4. A process according to claim 2 wherein said isoflavone material is separated from said soy molasses material at a temperature of about 0°C to about 10°C.
5. A process according to claim 1 further comprising forming an aqueous mixture of soy molasses material having a solids content of from about 3% to about 28% prior to separating said isoflavone enriched material from said soy molasses material.
6. A process according to claim 1 wherein said isoflavone enriched material is separated from said soy molasses material by centrifugation.
7. A process according to claim 6 wherein said centrifugation is performed while said soy molasses material is at a temperature of about 0°C to about 10°C and a pH of about 3 to about 6.5.
8. A process according to claim 1 wherein said isoflavone enriched material is separated from said soy molasses material by filtration.
9. A process according to claim 8 wherein said filtration is performed while said soy molasses material is at a temperature of about 0°C to about 10°C and a pH of about 3 to about 6.5.
10. A process according to claim 1 further comprising:
  - forming an aqueous slurry of said separated isoflavone enriched material, said isoflavone enriched material containing isoflavone conjugates; and
  - treating said slurry at a temperature and a pH for a sufficient time period to convert said isoflavone conjugates in said slurry to isoflavone glucosides to form an isoflavone glucoside enriched slurry.

11. A process according to claim 10 wherein said aqueous slurry of isoflavone enriched material has a solids content of about 6% to about 13%.
- 5 12. A process according to claim 10 wherein said slurry is treated at a pH of about 6 to about 13.5 and a temperature of about 2°C to about 120°C for a period of about 15 minutes to about 6 hours to convert said isoflavone conjugates to isoflavone glucosides.
- 10 13. A process according to claim 12 wherein said slurry is treated at a pH of about 9 to about 11 at a temperature of about 5°C to about 75°C for a period of about 15 minutes to about 6 hours to convert said isoflavone conjugates to isoflavone glucosides.
14. A process according to claim 10 further comprising separating an isoflavone glucoside enriched material from said slurry.
- 15 15. A process according to claim 14 wherein said isoflavone glucoside enriched material is separated from said slurry at a pH of about 3 to about 6.5 and at a temperature of about 0°C to about 35°C.
16. A process according to claim 15 wherein said isoflavone glucoside enriched material is separated from said slurry at a temperature of about 0°C to about 10°C.
- 20 17. A process according to claim 10 further comprising, after converting said isoflavone conjugates to said isoflavone glucosides, contacting an enzyme with said isoflavone glucosides in said slurry at a temperature and a pH for a time period sufficient to convert said isoflavone glucosides to aglucone isoflavones to form an aglucone isoflavone enriched slurry.
- 25 18. A process according to claim 17 wherein said enzyme is contacted with said isoflavone glucosides in said slurry at a pH of about 3 to about 9 and at a temperature of about 5°C to about 75°C for at least about 1 hour.
- 30 19. A process according to claim 17 wherein contacting an enzyme with said isoflavone glucosides in said slurry comprises adding an effective amount of a supplemental enzyme to said slurry.
20. A process according to claim 19 wherein said supplemental enzyme comprises a saccharidase enzyme capable of cleaving 1,4-glucoside bonds.
- 35 21. A process according to claim 17 further comprising separating an aglucone isoflavone enriched material from said slurry.
22. A process according to claim 21 wherein said aglucone isoflavone enriched material is separated from said slurry at a pH of about 3 to about 6.5 and at a temperature of about 0°C to about 35°C.
- 40 23. A process according to claim 22 wherein said aglucone isoflavone enriched material is separated from said slurry at a temperature of about 0°C to about 10°C.
24. A process for obtaining an isoflavone glucoside enriched material from soy molasses containing isoflavone conjugates, comprising:
- 45     treating said soy molasses at a pH and a temperature effective to convert isoflavone conjugates to isoflavone glucosides for a time period sufficient to convert said isoflavone conjugates to isoflavone glucosides; and
- 50     separating an isoflavone glucoside enriched material from said soy molasses.
25. A process according to claim 24 wherein said slurry is treated at a pH of about 6 to about 13.5 and at a temperature of about 2°C to about 120°C to convert said isoflavone conjugates to isoflavone glucosides.
- 55 26. A process according to claim 25 wherein said soy molasses is treated at a pH of about 9 to about 11 and at a temperature of about 5°C to about 75°C for a period of at least about 15 minutes to convert said isoflavone conjugates to isoflavone glucosides.

27. A process according to claim 26 wherein said soy molasses is treated at a pH of about 11 and a temperature of about 5°C to about 50°C for a period of about 15 minutes to about 1 hour to convert said isoflavone conjugates to isoflavone glucosides.
- 5       28. A process according to claim 27 wherein said soy molasses is treated at a pH of about 11 and at a temperature of about 35°C for about 30 minutes to about 1 hour to convert said isoflavone conjugates to isoflavone glucosides.
- 10      29. A process according to claim 26 wherein said soy molasses is treated at a pH of about 9 and a temperature of about 45°C to about 75°C for about 4 hours to about 6 hours to convert said isoflavone conjugates to isoflavone glucosides.
- 15      30. A process according to claim 24 wherein said isoflavone glucoside enriched material is separated from said soy molasses at a pH of about 3 to about 6.5 and a temperature of about 0°C to about 35°C.
- 15      31. A process according to claim 30 wherein said isoflavone glucoside enriched material is separated from said soy molasses material at a temperature of about 0°C to about 10°C.
- 20      32. A process according to claim 30 wherein said isoflavone glucoside enriched material is separated from said soy molasses material at a pH of about 4.5.
- 20      33. A process according to claim 30 wherein said isoflavone glucoside enriched material is separated from said soy molasses by centrifugation.
- 25      34. A process according to claim 30 wherein said isoflavone glucoside material is separated from said soy molasses by filtration.
35. A process according to claim 24 further comprising:
- 30      forming an aqueous slurry of said separated isoflavone glucoside enriched material; and
- 30      contacting an enzyme with said isoflavone glucosides in said slurry at a pH and a temperature for a time sufficient to convert said isoflavone glucosides to aglucone isoflavones to form an aglucone isoflavone enriched slurry.
- 35      36. A process according to claim 35 wherein said enzyme is contacted with said isoflavone glucosides in said slurry at a pH of about 3 to about 9 and at a temperature of about 5°C to about 75°C for at least about 1 hour.
- 40      37. A process according to claim 35 wherein contacting an enzyme with said isoflavone glucosides in said slurry comprises adding an effective amount of a supplemental enzyme to said slurry.
- 40      38. A process according to claim 37 wherein said supplemental enzyme comprises a saccharaidase enzyme capable of cleaving 1,4-glucoside bonds.
- 45      39. A process according to claim 35 further comprising, after converting said isoflavone glucosides to aglucone isoflavones, separating an aglucone isoflavone enriched material from said slurry.
- 45      40. A process according to claim 39 wherein said aglucone isoflavone enriched material is separated from said slurry at a pH of about 3 to about 6.5 and at a temperature of about 0°C to about 35°C.
- 50      41. A process according to claim 40 wherein said aglucone isoflavone enriched material is separated from said slurry at a temperature of about 0°C to about 10°C.
- 55      42. A process of obtaining an aglucone isoflavone enriched material from soy molasses containing isoflavone conjugates, comprising:
- 55      treating said soy molasses at a pH and a temperature for a period of time sufficient to convert said isoflavone conjugates to isoflavone glucosides;

contacting an enzyme with isoflavone glucosides in said soy molasses at a pH and a temperature for a time sufficient to convert said isoflavone glucosides to aglucone isoflavones; and

separating an aglucone enriched isoflavone material from said soy molasses.

- 5            43. A process according to claim 42 further comprising forming an aqueous mixture of soy molasses having a solids content of from about 3% to about 28% prior to converting said isoflavone conjugates to isoflavone glucosides.
- 10          44. A process according to claim 42 wherein said soy molasses is treated at a pH of about 6 to about 13.5 and a temperature of about 2°C to about 120°C for a period of at least about 15 minutes to convert said isoflavone conjugates to isoflavone glucosides.
- 15          45. A process according to claim 44 wherein said soy molasses is treated at a pH of about 9 to about 11 and at a temperature of about 5°C to about 75°C for a period of about 15 minutes to about 6 hours to convert said isoflavone conjugates to isoflavone glucosides.
- 20          46. A process according to claim 45 wherein said soy molasses is treated at a pH of about 11 and at a temperature of about 5°C to about 50°C for a period of about 30 minutes to about 1 hour to convert said isoflavone conjugates to isoflavone glucosides.
- 25          47. A process according to claim 42 wherein said enzyme is contacted with said isoflavone glucosides in said soy molasses at a pH of about 3 to about 9 and a temperature of about 5°C to about 75°C for at least about 1 hour.
- 30          48. A process according to claim 47 wherein said enzyme is contacted with said isoflavone glucosides in said soy molasses at a temperature of about 35°C to about 60°C.
- 35          49. A process according to claim 42 wherein contacting an enzyme with said isoflavone glucosides in said soy molasses comprises adding an effective amount of a supplemental enzyme to said soy molasses.
- 40          50. A process according to claim 49 wherein said supplemental enzyme comprises a saccharidase enzyme capable of cleaving 1,4 glucoside bonds.
- 45          51. A process according to claim 49 wherein the supplemental enzyme is selected from a group comprising alpha-glucosidase enzymes, beta-glucosidase enzymes, beta-galactosidase enzymes, gluco-amylase enzymes, pectinase enzymes, and combinations thereof.
- 50          52. A process according to claim 42 wherein said aglucone isoflavone enriched material is separated from said soy molasses at a pH of about 3 to about 6.5 and at a temperature of about 0°C to about 35°C.
- 55          53. A process according to claim 52 wherein said aglucone isoflavone enriched material is separated from said soy molasses at a temperature of about 0°C to about 10°C.
- 60          54. A process according to claim 52 wherein said aglucone isoflavone enriched material is separated from said soy molasses at a pH of about 4.5
- 65          55. A process according to claim 52 wherein said aglucone isoflavone enriched material is separated from said soy molasses by centrifugation.
- 70          56. A process according to claim 52 wherein said aglucone isoflavone enriched material is separated from said soy molasses by filtration.
- 75          57. An isoflavone enriched material separated from soy molasses.
- 80          58. An isoflavone glucoside enriched material separated from soy molasses.
- 85          59. An aglucone isoflavone enriched material separated from soy molasses.



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## EUROPEAN SEARCH REPORT

Application Number  
EP 97 30 4076

DOCUMENTS CONSIDERED TO BE RELEVANT									
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)						
X,Y	BARNES S ET AL: "ISOFLAVONES AND THEIR CONJUGATES IN SOY FOODS: EXTRACTION CONDITIONS AND ANALYSIS BY HPLC-MASS SPECTROMETRY" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 42, no. 11, November 1994, pages 2466-2474, XP000474641 see in particular page 2467, materials and methods * the whole document * ---	1-59	C07D311/40 C07D311/36 C07H17/07						
X,Y	COWARD L ET AL: "GENISTEIN, DAIDZEIN, AND THEIR BETA-GLYCOSIDE CONJUGATES: ANTITUMOR ISOFLAVONES IN SOYBEAN FOODS FROM AMERICAN AND ASIAN DIETS" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 41, no. 11, November 1993, pages 1961-1967, XP002028058 see in particular experimental procedures * the whole document *	1-59							
Y	DATABASE WPI Section Ch, Week 8947 Derwent Publications Ltd., London, GB; Class B03, AN 89-345164 XP002041931 & JP 01 258 669 A (KIKKOMAN CORP) , 16 October 1989 * abstract *	1-59	C07D C07H						
<p>The present search report has been drawn up for all claims</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Place of search</td> <td style="width: 33%;">Date of completion of the search</td> <td style="width: 33%;">Examiner</td> </tr> <tr> <td>MUNICH</td> <td>26 September 1997</td> <td>Steendijk, M</td> </tr> </table>				Place of search	Date of completion of the search	Examiner	MUNICH	26 September 1997	Steendijk, M
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MUNICH	26 September 1997	Steendijk, M							
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document							
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document									



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Application Number  
EP 97 30 4076

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P,Y	DATABASE WPI Section Ch, Week 9702 Derwent Publications Ltd., London, GB; Class B02, AN 97-017368 XP002041933 & JP 08 283 283 A (KIKKOMAN CORP) , 29 October 1996 * abstract *	1-59		
E	WO 97 26269 A (ACDS TECHNOLOGIES INC) 24 July 1997 * the whole document *	1-59	<table border="1"> <tr> <td>TECHNICAL FIELDS SEARCHED (Int.Cl.6)</td> </tr> </table>	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
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<p>The present search report has been drawn up for all claims</p>				
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